REMARKS

Claims 22-37 are pending. Claims 1-6 are hereby cancelled without prejudice to the prosecution of their subject matter in subsequent applications. New claims 22-37 are added, and do not consitute new matter.

Claims 1-6 are rejected under 35 U.S.C. § 112, first paragraph, for allegedly lacking enablement. Claims 1-6 are further rejected under 35 U.S.C. § 103(a), as being allegedly unpatentable over Hoch *et al.* (U.S. Patent No. 6,043,045) in view of Dasgupta *et al.* (Tubercle and Lung Disease (2000): 80(3) 141-159). For reasons set forth below, it is respectfully requested that the rejections be withdrawn and that the claims be expediently allowed.

The Invention

The present invention relates to screening methods intended to identify drugs that may be useful against pathogenic microbes and focuses particularly on the two-component systems of DevR-DevS and DevR-Rv2027c. Specifically, the invention comprises a method of over-expressing DevR, DevS and Rv2027c, autophosphorylating the histidine sensor kinase of the DevS or Rv2027c protein, and transferring the phosphate entity to DevR via a phosphotransfer event. This cascade of events results in a change in binding potential of the regulatory response protein to its DNA target, thereby effecting a change in the expression of genes under its regulatory control. The screening method of the present invention thus possesses the potential to regulate dormancy of bacili dependent upon the function of these two-component systems and may facilitate

the development of an antibiotic useful in the treatment of conditions such as tuberculosis.

The Claims Are Supported By The Written Description

Claims 1-6 are rejected under 35 U.S.C. § 112, first paragraph, because the claims allegedly contain subject matter that is not described in the specification in such a way as to enable one of ordinary skill in the art to reproduce the invention. Specifically, the Examiner asserts that the recitation of use of an "SDS-PAGE based high throughput assay" in claim 1 is not supported by the disclosure. Amendment of claim 1 to reflect the use of ***either*** SDS-PAGE based ***or*** high throughput assays remedies this rejection. Additionally, the Examiner alleges that the appearance of the term "DevB" in claim 1 lacks support in the disclosure. The new claims obviate the basis of this rejection.

The Examiner further asserts that the language of claim 1, section c, part ii, in which it is stated that "the degree of phosphotransfer from phosphorylated DevS, and Rv2027 proteins to DevR" is inversely proportional to the drug potential of the test compound, is not supported by the specification. Applicants respectfully disagree. Paragraphs [0001], [0059] and [0064] of the disclosure clearly state that drug potential is inversely proportional to "the degree of dephosphorylation of phosphorylated species of DevS and Rv2027c," thereby providing unambiguous support for the language of claim 1. Further, Figure 9 of the present invention provides additional support for the language of claim 1, section c, part ii.

Finally, the Examiner indicates that the specification does not support claim 1, section c, part iii, which states that the drug potency is inversely proportional to "the degree of loss of phosphate-associated radioactivity from DevS/Rv2027c and DevR in a reaction containing DevS, DevR/Rv2027 and DevR." However, paragraph [0177] of the specification clearly states that "addition of DevR...protein to phosphorylated sensor kinase and subsequent incubation...led to a net reduction in retention of radiolabel...in the sensor kinase/s due to phosphotransfer and subsequent dephosphorylation reaction of DevR." Therefore, the specification clearly supports the language of claim 1, section c, part iii. Further, when there is a loss of phosphate-associated radioactivity in a reaction containing DevS/Rv2027 and DevR, it is evident from the experimental results as represented in Figure 15 that a test compound with inhibitory activity would prevent the loss of radioactivity from the phosphorylated DevR protein, and further that the degree of loss would be inversely proportional to the potency of the drug compound being tested. Based on the foregoing reasoning, there is clear support for the language of claim 1 in the specification. Applicants thus respectfully request that the rejection of claims 1-6 under 35 U.S.C. § 112 be withdrawn and the claims allowed.

The Claims Are Not Obvious

Claims 1-6 are rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Hoch *et al.* (U.S. Patent No. 6,043,045; referred to henceforth as "the '045 patent") in view of Dasgupta *et al.* (Tubercle and Lung Disease (2000), 80(3):141-159; referred to henceforth as "Dasgupta"). The Examiner alleges that (1) the '045 patent discloses a high-throughput screen for identifying new antibiotic, antibacterial, or

antimicrobial drug agents that inhibit bacterial two-component systems using methodology based upon levels of phosphorylation and radioactivity as measured by SDS-PAGE; and (2) Dasgupta discloses the DevR-DevS two component system in Mycobacteria and homology of Rv2027c to DevS. It is further alleged that one of skill in the art would be motivated to apply the assay of the '045 patent to the DevR-DevS/Rv2027c two-component systems due to the shared phosphorylation reactions disclosed in both references. The Examiner further asserts that the relationship anticipated between drug efficacy and phosphotransfer or radioactivity is obvious in light of the above-mentioned references.

Applicants respectfully traverse the rejection. Preliminarily, the Examiner asserts that Dasgupta's disclosure of the DevR-DevS two-component system in mycobacteria and the homology of Rv2027c with DevS obviates the presence of the specific phosphorylation properties of the present invention. However, the mere identification of the homology of Rv2027c with DevS does not confirm the presence of similar characteristics such as phosphorylation properties. The reference in Dasgupta to Rv2027c sensor kinase homology refers only to preliminary expression studies that merely suggest that Rv2027c might play a role similar to that of DevS. Moreover, Dasgupta merely predicted the catalytic site of the DevR-DevS molecules utilizing bioinformatics tools and did not offer conclusive proof of their location or function. Contrary to the assertions of the Examiner, the sizes of the relevant transcripts were not determined; rather, only co-transcription of *devR* and *devS* was established. Also unproven by Dasgupta is a linear relationship between transcript size and protein length.

As such and based on the incomplete understanding of the DevR-DevS/Rv2027c two-component systems demonstrated in the '045 patent, the present invention cannot be said to be obviated by the '045 patent in view of Dasgupta.

Additionally, the methods disclosed by the '045 patent differ substantially from those utilized in the present invention, providing further support for a finding of nonobviousness. The Examiner states that "the use of SDS-PAGE with multiple samples can be interpreted as 'SDS-PAGE based high throughput assaying'" (page 4, paragraph 1 of the instant Office Action). However, this is not necessarily the case due to the limitation of the number of samples that can be analyzed and then time required to obtain quantitative results. The '045 patent expressly mentions this fact in column 2, lines 34-35. Moreover, the Examiner's assertion that the radioactivity remaining on the resin is measured is incorrect; rather, the content of each well ***except for*** the resin was collected into a 96-well MicroFluor plate and the filtered contents of the well were counted by the radioactive counter (column 18, lines 33-39). Therefore, the interpretation of the method used in the '045 patent is incorrect and it cannot be said that the present invention is rendered obvious by the '045 patent.

Additionally, the '045 patent utilizes KinA and Spo0F proteins, and fails to disclose or suggest the application of the claimed experimental technique to the DevR-DevS/Rv2027c two-component systems. The phosphotransfer assay described in the '045 patent is a multistep assay developed specifically to accommodate the particular features of the KinA-Spo0F two-component system. Purified Spo0F is first immobilized to Ni-Resin (column 17, lines 49-61), followed by phosphorylation of bound His-Spo0F,

kinase removal, Spo0F elution, collection of eluted phosphorylated His-Spo0F by filtration, and either SDS-PAGE or acid hydrolysis of phosphorylated Spo0F followed by filtration. The amount of reaction product formed is determined by quantifying the inorganic phosphate recovered from the filtrate.

In contrast to the assays described by the '045 patent, the present invention does not require assays in which the substrates (DevR, DevS, or Rv2027c) are immobilized on a solid support. Instead, autophosphorylation and phosphotransfer reactions can be carried out in solution. In addition, the present invention enables a single step high throughput assay wherein the soluble reaction products are discarded by filtration and the radiolabels are retained on the filter in association with the proteins and quantified by counting the filters themselves. The unique kinetics of the DevR-DevS/Rv2027c system facilitates the use of a coupled assay in a filter-based format without the need to separate the kinase from the substrate. This improvement over the prior art based upon the distinct characteristics of the DevR-DevS/Rv2027c two-component system is not taught or suggested by either the '045 patent or Dasgupta and thus is not rendered obvious by the combination of these references.

For the reasons set forth above, Applicants respectfully suggest that the new claims are not obvious over the cited references and the rejections under 35 U.S.C. § 103(a) should be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully request withdrawal of the outstanding rejections and allowance of the pending claims. Should any additional fee be required, or if any overpayment has been made, the Commissioner is hereby authorized to charge any fees, or credit any overpayments made, to Deposit Account 02-4377.

Respectfully submitted,

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By: _____

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